Early Journal Content on JSTOR, Free to Anyone in the World

This article is one of nearly 500,000 scholarly works digitized and made freely available to everyone in the world by JSTOR.

Known as the Early Journal Content, this set of works include research articles, news, letters, and other writings published in more than 200 of the oldest leading academic journals. The works date from the mid-seventeenth to the early twentieth centuries.

We encourage people to read and share the Early Journal Content openly and to tell others that this resource exists. People may post this content online or redistribute in any way for non-commercial purposes.

Read more about Early Journal Content at http://about.jstor.org/participate-jstor/individuals/early-journal-content.

JSTOR is a digital library of academic journals, books, and primary source objects. JSTOR helps people discover, use, and build upon a wide range of content through a powerful research and teaching platform, and preserves this content for future generations. JSTOR is part of ITHAKA, a not-for-profit organization that also includes Ithaka S+R and Portico. For more information about JSTOR, please contact support@jstor.org.

ON SOME CULTURAL RELATIONS AND ANTAGONISMS OF BACILLUS COLI AND HOUSTON'S SEWAGE STREPTOCOCCI; WITH A METHOD FOR THE DETECTION AND SEPARATION OF THESE MICRO-ORGANISMS IN POLLUTED WATERS.*

S. C. Prescott and S. K. Baker.

(*From the Biological Laboratories, Massachusetts Institute of Technology, Boston.*)

It is generally believed that the occurrence of B. coli in considerable numbers in water supplies is an indication of fecal pollution and renders the water unsuitable for drinking purposes. The opinion that this organism is characteristic of pollution from human sources was long since proved to be erroneous, as it was shown by Dyar and Keith (1), Smith (2), Flint (2A), Belitzer (3), and Moore and Wright (4), to be constantly present in the intestines of many groups of animals. More recently a number of investigators in Europe and America have reported that bacilli behaving in all respects like the colon bacillus of the human intestine are frequently found in nature where there is no evidence of direct or immediate fecal pollution. Kruse (5) and Weissenfeld (6) declared that B. coli is present in almost all waters, good or bad. One of us (7, 8) has isolated from grains and products of milling a considerable number of organisms having all the characteristics of B. coli, and Papasotiriu (9) obtained the same results in Europe in an investigation of similar scope. Other workers have also published results which lead to the conclusion that bacteria corresponding in every way to B. coli are by no means confined even to animal intestines, but are widely distributed in nature. While this fact has not yet destroyed altogether the value of the "colon test" for potable waters, it has made it clear that extreme care should be used in interpreting the results thereby obtained, as has already been pointed out by Freudenreich (10), Smith (11), and one of us (12); and it has also led to a belief in some quarters that, if possible,

---

*Received for publication November 6, 1903.

confirmatory evidence of some sort should be adduced whereby the bacteriologist may establish beyond doubt recent intestinal pollution.

Such a means of confirmation seems to be offered by the streptococci of Houston, which have been shown by various workers to be present in sewage, sewage effluents, and polluted waters, and in soil which has received the wastes of animal life, but to be absent from unpolluted waters and virgin soils. Originally reported by Laws and Andrewes (12) in 1894, the importance of these micro-organisms was not emphasized until 1899 and 1900, when Houston (13) laid special stress upon the fact that streptococci and staphylococci seem to be characteristic of sewage and animal waste, the former being, in his opinion, the more truly indicative of recent pollution. Horrocks (14) found these organisms in great abundance in sewage, and in waters known to be polluted but which contained no traces of B. coli. He found by experiment that B. coli gradually disappeared from many specimens of sewage kept in the dark at the temperature of an outside veranda. But the commonest forms which persisted were varieties of streptococci and staphylococci.

The first investigators in this country to call attention to these streptococci were Winslow and Miss Hunnewell (15) in 1901. They first observed them in the washings from the hands of school children, and have since isolated them from crude sewage and from many polluted waters. They found that in Smith fermentation tubes "dextrose-fermenting organisms, which the immediate inoculation of the dextrose broth showed to be present . . . . in forty-eight samples, disappeared in forty-four cases during the two incubations of twenty-four hours each." On analysis, the persisting organisms were found to be streptococci or staphylococci which had overgrown or killed out the colon bacilli in nearly every case. In only three out of 157 samples of unpolluted waters, however, did they find streptococci when 1 c.c. of the water was examined.

A note by one of the present writers (16) in 1902 set forth the difficulty of making pure cultures of B. coli from inoculations of dextrose broth with fecal matter, if the incubation is carried too far. It was found that other organisms, especially streptococci, develop abundantly and overgrow the colon bacilli, which are present in chief during the period of gas-formation.

Gage (17), at the Lawrence Experiment Station, has reported the streptococci present in the sewage of that city, while LeGros (18), in a recently published monograph describes many streptococci, all isolated either from the body or from sewage. It seems, indeed, from all existing evidence, that the streptococci are, as a class, closely associated with animal bodies, occurring either on the surface or within the intestinal tract. It seems reasonable, therefore, to regard these organisms as indicative of recent pollution, as suggested by Houston.

Since, as we now know, B. coli is widespread in nature, it may perhaps be present in considerable numbers in waters otherwise unobjectionable, although we have as yet no proof of this possibility. It is also conceivable that streptococci may be found in pure water, though all evidence at the present time seems to render this improbable. But it is at present very unlikely that both forms will occur abundantly at the same time in any unpolluted waters, while, on the other hand, we know that both are abundant in sewage. It will therefore obviously be of advantage in judging the quality of a water if the presence of both kinds of bacteria can be demonstrated in it.

With this end in view we have recognized the desirability of examining waters for both colon bacilli and streptococci, and have attempted to devise a method whereby this examination may be conducted with sufficient ease, quickness, and certainty to become a useful "laboratory test." For this purpose we have made use of some of the cultural reactions of the two organisms, especially those with dextrose and other sugar broths, and litmus lactose agar. Differences are also manifest in appearance, in gelatin and agar tube cultures, in the effect produced in nitrate and indol solutions, and on potato. A more comprehensive comparison of the two organisms may be made from the descriptions below:

CHARACTERISTICS OF COLON BACILLI AND SEWAGE STREPTOCOCCI.

	COLON BACILLI.	SEWAGE STREPTOCOCCI.
FORM.	*Bacillus* 2–3μ long by 0.5μ wide; Rounded ends.	*Coccus* 1μ in diameter.
GROUPING.	Occurs singly, or in short chains or masses.	Occurs in short chains, often in pairs.
MOTILITY.	Actively motile.	Non-motile.
SPORE FORMATION.	None.	None.
GELATIN PLATE.	Thin, irregular film. Much as on agar, colonies smaller.	Colonies similar to those on agar; no liquefaction.
GELATIN STICK.	Three days: transparent nail growth; gelatin not liquefied.	Three days: nail growth, apparently made up of isolated colonies; very slight spreading on surface.
AGAR PLATE.	Twelve hours: surface colonies opalescent, nearly circular, edges smooth. Submerged colonies clear cut, lenticular. Two days: surface colonies take irregular vine-leaf form.	Colonies small; under a low power somewhat irregular in shape; edges smooth.
AGAR SLOPE.	Twenty-four hours; luxuriant, moist, opalescent, translucent, white growth, narrowing from bottom to top.	Twenty-four hours: faint dotted growth.
NUTRIENT BROTH.	Twelve hours: distinct turbidity. Eighteen hours: sediment. Two days: no scum on surface.	Eighteen hours; turbidity and perceptible sediment; on shaking, sediment rises in spiral.
LITMUS MILK.	Litmus reddened, and then decolorized in twelve hours: milk coagulated in eighteen hours.	Twelve hours: slightly acid; litmus slightly decolorized. Eighteen hours: strongly acid. Thirty-six hours: milk coagulated.

POTATO.

Luxuriant, dirty yellow growth; potato not discolored.	Hardly perceptible white growth after three days.

DEXTROSE BROTH.

Twelve hours: dextrose fermented with formation of acid and gas.	Eighteen hours: strongly acid; no gas; sediment and turbidity in both arms.

SACCHAROSE BROTH.

Twelve hours: saccharose fermented with formation of acid and some gas.	Eighteen hours: turbidity and sediment, but no evidence of change in sugar.

LACTOSE LITMUS AGAR PLATE.

Litmus reddened in twenty-four hours; bubbles of gas formed.	Twelve hours: litmus reddened; colonies small, with slight pink tint as if colored by litmus.

PEPTONE AND SALT (DUNHAM).

Three days: pronounced nitroso-indol reaction.	Apparently no growth.

ANAËROBIC AGAR SLOPE.

Thin, transparent growth, much less strong than in aërobic streak.	Dotted growth, less strong than in aërobic culture.

Our preliminary experiments were based on the results of Winslow and Miss Hunnewell, and of one of the writers in the other papers mentioned. In view of our results, it seemed probable that there would be little difficulty in establishing a time relation between the growth periods of the two organisms in dextrose broth which would allow the isolation of both from the same culture if both were originally present in the sample used for inoculation. The soundness of this conclusion was first tested experimentally by the use of mixtures in known ratios of the two organisms in question, the culture used for this purpose being isolated from fresh feces by inoculation into dextrose broth, and plating out upon litmus lactose agar after six and forty-eight hours incubation at 37°C.

The method was as follows: Two bottles of sterile water, containing 100 c.c. each, were inoculated, one with B. coli, the other with streptococci, and plates made from these to determine the number of each kind of bacteria used. With the water suspensions thus prepared mixtures were made, of various dilutions and proportions, of B. coli and streptococci. From each of these mixtures several Smith fermentation tubes containing dextrose broth (meat juice with 1 per cent. peptone and 2.5 per cent. dextrose) were inoculated with 1 c.c. each. The dextrose tubes were then incubated at 37° C., and litmus lactose agar plates made from them at various intervals. In plating, 1 c.c. of the dextrose broth culture was diluted in sterile water to approximately 1:100,000; 1:1,000,000; or 1:10,000,000; and 1 c.c. of this dilution inoculated into plates containing lactose agar to which sterile neutral litmus had been added. These plates were incubated at 37° C. for twenty-four hours, and then allowed to stand for some time at the room temperature before counting.

The colonies of B. coli and streptococci were distinguished by their different tints in the litmus lactose agar plate, and by further differences in shape and general appearance. The submerged colon colonies generally appeared of an oval or lenticular shape, and were not strongly acid. The streptococci, on the other hand, were most frequently spherical or nearly so, and became much more intensely reddened. After a little practice the two kinds of organisms could be detected without difficulty. In order to avoid error in this respect, however, cover-glass preparations from the separate colonies were examined microscopically for the morphology of the bacteria throughout the early part of the investigation.

The results obtained varied widely, as was to be expected, according to the number and proportions of the organisms inoculated. There was, however, in every case a period during which the colon bacilli were abundant in the dextrose tubes, while the streptococci were indistinguishable or nearly so. This condition was followed by a decrease in the number of B. coli and a sudden increase in streptococci, the colon bacilli at length becoming imperceptible and the streptococci rising to a maximum at which they persisted for a considerable time. The following tables show the numbers inoculated and the numbers found in the tubes at different platings:

TABLE I.

SERIES I.

	TUBE NUMBERS				
	1	2	3	4	5
Ratio—streptococci to B. coli inoculated........	1:10	1:5	3:10	1:20	1:30
No. of B. coli inoculated ...	145,000	145,000	145,000	290,000	435,000
No. of streptococci inocul't'd	14,500	29,000	43,500	14,500	14,500
Millions per c.c. of colon bacilli present after 9 hrs.	5.5	6.8	10	12	12.5
Streptoc'i pres'nt after 9 hrs.	0	0	0	0	0

NOTE.—This was a preliminary series to determine dilutions to be used and the general behavior of the bacteria. It was only possible to count the first plating.

The colonies of B. coli and streptococci were distinguished by the different tint in the litmus lactose agar plate, and by differences in shape and appearance. To be perfectly certain, however, cover-glass preparations from the separate colonies were examined microscopically.

SERIES II.

	TUBE NUMBERS				
	6	7	8	9	10
Ratio—streptococci to B. coli inoculated....	1:16	1:8	1:5	1:32	1:50
No. of B. coli inoculated	100,000	100,000	100,000	200,000	300,000
No. of streptococci inocul't'd	6,000	12,000	18,000	6,000	6,000
No. found in tubes (millions per c.c.) after—					
18 hours, B. coli	600	500	440	420	410
Streptococci...........	0	0	0	0	0
27 hours[1]	400	600	500	450	300
42 hours, B. coli	0	0	0	...	0
Streptococci...........	62	120	135	...	154
51 hours, B. coli	0	0	0	0	0
Streptococci...........	60	100	115	90	85
65 hours, B. coli	0	0	0	0	0
Streptococci...........	70	65	35	80	80
95 hours, B. coli	0	0	0	0	0
Streptococci...........	90	70	48	85	90

[1] Proportions indeterminate.

SERIES III.

	Tube Numbers		
	11	12	13
Ratio—streptococci to B. coli inoculated	21:1	210:1	105:1
No. of B. coli inoculated	2,250	225	450
No. of streptococci inoculated	47,000	47,000	47,000
No. found in tubes (millions per c.c.) after—			
9 hours, B. coli	Many[2]	Many[2]	Many[2]
Streptococci	0	0	0
12 hours, B. coli	400	260	100
Streptococci	100	240	210
14 hours, B. coli	360	...	290
Streptococci	110	...	260
24 hours, B. coli	30	50	0
Streptococci	270	450	370
33 hours, B. coli	110	0	0
Streptococci	230	500	274
50 hours, B. coli	0	0	0
Streptococci	280	280	285
77 hours, B. coli	0	0	0
Streptococci	270	400	400

SERIES IV.

	Tube Numbers		
	14	15	16
Ratio—streptococci to B. coli inoculated	1:100	1:1	100:1
No. of B. coli inoculated	160,000	1,600	1,600
No. of streptococci inoculated	1,700	1,700	170,000
No. found in tubes (millions per c.c.) after—			
11 hours, B. coli	350	320	300
Streptococci	0	0	0
14 hours, B. coli	640	350	200
Streptococci	30	70	170
19 hours, B. coli	400	340	32
Streptococci	110	104	124
24 hours, B. coli	494	354	74
Streptococci	80	96	284
33 hours, B. coli	346	112	0
Streptococci	100	58	280
41 hours, B. coli	3	0	0
Streptococci	60	83	147
67 hours, B. coli	0	0	0
Streptococci	6.2	0.18	0.51
85 hours, B. coli	0	0	...
Streptococci	0.22	0.22	...
Gas formation begun after	6 hours	9 hours	10 hours

---

[2] Count not made.

SERIES V.

	Tube Number	
	17	18
Ratio-streptococci to B. coli inoculated	1:2	53:1
No. of B. coli inoculated	32	3
No. of streptococci inoculated	16	160
No. in tube (in millions per c.c.) after—		
11 hours, B. coli	2.5	7.6
Streptococci	0.3	0.3
25 hours, B. coli	160	554
Streptococci	380	100
37 hours, B. coli	0.1	194
Streptococci	200	60
43 hours, B. coli	0	0
Streptococci	300	83
62 hours, B. coli	0	0
Streptococci	268	61
Gas formation begun after	15 hrs.	16 hrs.

To facilitate the systematic study of these results, we insert the following table, showing the time of approach of B. coli to a maximum, the time of appearance of streptococci, that of the approximate disappearance of B. coli, and that of the first formation of gas, in the few cases in which it was recorded.

TABLE II.

Tube Number	B. coli Inoculated	Streptococci Inoculated	First Gas Formed	B. coli Maximum	Appearance of Streptococci	Disappearance of B. coli
16	1,600	170,000	After 10 hrs.	After 11 hrs.	After 14 hrs.	After 33 hrs.
11	2 250	47,000	..	12	12	50
13	450	47,000	..	10	12	24
12	225	47,000	..	12	12	33
8	100,000	18,000	..	18	..	42
7	100,000	12,000	..	18	..	42
9	200,000	6,000	..	18	..	42
10	300,000	6,000	..	18	..	42
14	160,000	1,700	6	14	14	67
15	1,600	1,700	9	11	14	41
17	3	160	16	25	11	43
18	32	16	15	25	11	43

This table seems to indicate that, while the time of appearance of gas varies inversely with the numbers of B. coli originally present, the colon maximum and the appearance of streptococci vary inversely with the number of streptococci first used, and the disappearance of the B. coli with the ratio of streptococci to colon bacilli. Some of the variations we are at a loss to explain, but it may be that further experiments will show that they were accidental.

EXAMINATION OF SEWAGE AND POLLUTED WATERS.

The results obtained with the mixture of known ratios gave promise of usefulness when the method was applied to actual examination of water and sewage, and in general the results have been much the same.

About fifty samples of waters have been examined. Each of these was tested quantitatively by making a suitable dilution, plating in litmus lactose agar, and incubating for twenty-four hours at 37°. The majority of the colonies thus obtained gave a well-marked acid reaction, but this was not specially recorded in the counting. At the time of plating 1 c.c. of each sample was inoculated into dextrose broth.

A preliminary series was first examined as a test of the method. The samples were as follows:

a) Crude sewage from Dartmouth street (Boston) sewer, diluted 1:100, April 22.
b) Charles River, at Otter street, April 22.
c) Charles River, at Exeter street, April 22.
d) Charles River, at Newton Lower Falls, April 30.
e) Charles River, at Pinckney street, April 30.

The results obtained by plating after various periods are shown in the accompanying tables.

The method proving fairly satisfactory, a systematic examination of forty-six samples of water was next undertaken. The number of bacteria present was determined in some cases by plating on gelatin, and the number of acid-producing colonies in litmus lactose agar was also usually noted.

From the tables it is apparent that the waters showed in general the same periodic changes as did the artificial mixtures: pure cultures of B. coli were first obtained in the plates, then the gradual displacement of one form by the other took place, and at length only streptococci or allied forms were discernible. In a few instances the results were somewhat obscured by the growth of a proteus-like organism which developed in the open arm of the dextrose broth tube, but in our opinion both B. coli and streptococci can be detected in water by this method in almost all cases when both organisms are present, provided the number of

TABLE III.

SERIES A.

	TUBE NUMBER		
	a	*b*	*c*
No. of bacteria inoculated	580,000	17,000	17,000
No. in tubes (millions per c.c.) after—			
11 hours, B. coli	274	100	280
Streptococci	0	0	0
14 hours	....	138	422
19 hours, B. coli	50	0	0
Streptococci	190	60	210
24 hours, B. coli	2	0	2
Streptococci	180	250	230
33 hours, B. coli	22	8	3
Streptococci	154	47	10
41 hours, B. coli	6	0	5
Streptococci	134	8	58
67 hours, B. coli	0	0	0
Streptococci	40	51	23
85 hours, B. coli	0	0	0
Streptococci	11	37	19
Gas formation begun after	6 hrs.	8 hrs.	8 hrs.

SERIES B.

	TUBE NUMBER	
	d	*e*
No. of bacteria inoculated	1,200	9,000
No. found in tubes (millions per c.c.) after—		
11 hours, B. coli	3	16
Streptococci	0	0
25 hours, B. coli	8	20
Streptococci	0	300
35 hours, B. coli	..	0
Streptococci	..	200
43 hours, B. coli	0	0
Streptococci	175	178
Gas formation begun after	13 hrs.	10 hrs.

conflicting organisms does not greatly exceed that of the colon bacilli.

That certain definite cultural relations exist between colon bacilli and streptococci when grown together in dextrose broth, and when no conflicting organism is present in great abundance,

TABLE IV.

SERIES I.

	SAMPLE NUMBER									
	1	2	3	4	5	6	7	8	9	10
Total bacteria, gelatin count	250	25	123	271	300	64	45	65	8	33
Acid-producing bacteria, litmus lactose agar	4	10	9	5	8	55	35	460	1250	105
No. of bacteria (mill. per c.c.) after gr'th in dext. broth for—										
11 hours, B. coli	1	20	68.4	200	185	400	130	332	420	410
Streptococci	0	0	0	0	0	0	0	0	0	0
16 hours, B. coli	200	76	130	270	220	210	140	420	285	410
Streptococci	40	25	20	10	45	30	20	210	75	145
23 hours, B. coli	280	150	385	370	300	570	200	405	320	300
Streptococci	140	85	280	170	300	1700	110	350	370	350
39 hours, B. coli	0	0	25	110	0	210	20	24	105	...
Streptococci	474	420	480	300	390	170	400	105	250	...
63 hours, B. coli	0	0	0	0	0	12	8	0	0	0
Streptococci	2	0	0	45	1	2	45	150	86	170
First gas noted after (hrs.)	10	10	9	9	10	8	10	6	6	8

SERIES II.

	SAMPLE NUMBER						
	11	12	13	14	15	16	17
Gelatin count	20000	20000	...	...	...	...	...
Red colonies	0	6	0	0	0	1	8
No. of bacteria (mill. per c.c.) after gr'th in dext. broth for—							
8 hours, B. coli	1.2	11.4	6.8	6.7	6.9	8.5	0.5
Streptococci	0	0	0	0	0	0	0
22 hours, B. coli	300	500	350	450	440	358	2000
Streptococci	200	60	225	136	10	265	300
32 hours, B. coli	130	10	70	170	50	300	490
Streptococci	220	200	620	210	160	540	280
46 hours, B. coli	0	....	Few[3]	0	100	100	Few[3]
Streptococci	7200	....	Many	7000	3000	1000	Many
60 hours, B. coli	280	1	0	...	...	...	62
Streptococci	80	180	640	...	...	...	20

---

[3] Count inaccurate, owing to presence of conflicting organisms.

is evident from the results with waters, as well as with mixtures of known ratio. The colon bacilli invariably develop with greater rapidity than the streptococci under the cultural conditions which we have employed, but the latter finally overgrow or kill out the colon bacilli. This fact is probably due to the greater sensitiveness of B. coli to the lactic acid produced in the medium by the streptococci over and above that formed by the colon bacilli themselves.

SERIES III.

	SAMPLE NUMBER							
	18	19	20	21	22	23	24	25
Gelatine count	Not made							
Red colonies	1	150	25	30	50	170	200	30
No. of bacteria (mill. per c.c.) after gro'th in dext. br. for—								
7 hours, B. coli	0.02	...	...	0.01	0.04	0.12	0.55	1.1
Streptococci	0	...	...	0	0	0	0	0.01
17 hours, B. coli	266	100	88	350	510	380	330	160
Streptococci	150	0	40	140	240	128	80	220
27 hours, B. coli	520	610	72	700	1000	740	100	300
Streptococci	800	860	670	1080	2500	4380	0	3900
40 hours, B. coli	0	0	10	22	36	7	7.2	...
Streptococci	252	330	260	22	66	60	5.2	..
52 hours, B. coli	10	16	38	20	70	35	10	27
Streptococci	40	16	3.8	31	41	25	10	30

SERIES IV.

	SAMPLE NUMBER	
	26	27
Gelatin count	1000	940
Red colonies	1200	20
Number of bacteria (millions per c.c.) after growth in dextrose broth for—		
6 hours, B. coli Streptococci	Growths obscured by other organisms	
19 hours, B. coli	250	230
Streptococci	52	300
31 hours, B. coli	180	120
Streptococci	440	300
43 hours, B. coli	90	480
Streptococci	...	400
54 hours, B. coli	12	15
Streptococci	470	620

Under the favorable conditions at first existing, the colon bacilli develop very rapidly, fermenting the dextrose with the formation of gas and acid in from six to eighteen hours (the time varying according to the number of these organisms originally present), and attain a maximum in from twenty to thirty-six hours. The streptococci gradually increase along with the formation of larger quantities of lactic acid, until at the end of fifteen to twenty-four hours they become distinguishable in plates inoculated from the dextrose tube. At about the same time the colon bacilli apparently become unfavorably affected by the increase in acidity, and

SERIES V.

	Sample Number								
	28	29	30	31	32	33	34	35	36
Gelatin count	...	...	5	41	34	45	160	214	...
Red colonies	Not determined								
No. of bacteria (mill. per c.c.) after gr'th in dext. broth for—									
12 hours, B. coli	200	..[4]	..[4]	..[4]	120	120	3	50	300
Streptococci	...	...	...	...	0	0	0	4	20
24 hours, B. coli	100	380	370	410	400	330	...	80	360
Streptococci	900	60	380	620	500	140	...	150	180
36 hours, B. coli	290	50	80	20	50	20	440	20	60
Streptococci	50	150	440	280	340	1500	90	320	200
60 hours, B. coli	280	1	0	...	...	...	64	...	3
Streptococci	80	180	640	...	...	...	25	...	65

SERIES VI.

	Sample Number									
	37	38	39	40	41	42	43	44	45	46
Gelatin count	38	12	22	15	35	115	70	72	105	40000
Red colonies	16	2	2	2	31	6	13	16	15	1200
No. of bacteria (mill. per c.c.) after gr'th in dext. broth for—										
12 hours, B. coli	55	16	22	0	70	100	300	700	130	...[5]
Streptococci	0	0	0	0	0	0	0	0	10	....
24 hours, B. coli	360	Proteus		400	...	260	320	550	720	40
Streptococci	360			Few	...	140	170	300	500	200
36 hours, B. coli	0	0	70	25	..[5]	40	240	260	0	0
Streptococci	Many	500	300	75		100	100	180	Many	450
48 hours, B. coli	0	2	0	0	0	0	..[5]	0	0	0
Streptococci	2	6	3	40	1	26	...	9	10	7

---

[4] Conflicting organisms.

[5] Other organisms partly obscured the medium.

rapidly decrease until they are no longer perceptible. The increase in streptococci is rapid after they appear—so rapid, in fact, that small numbers of B. coli would hardly affect the efficiency of an isolation made a few hours before the colon bacilli disappear. The streptococci attain a maximum soon after the other organisms disappear, and remain near this maximum for one or two days. The time of incubation allowable before making plate cultures for isolation of streptococci has, therefore, wide limits.

The time of beginning of gas formation is a factor of considerable importance. In the few cases in which this time was noted with our mixtures of known ratio, the colon bacilli attained their maximum numbers within a few hours after gas formation began. In the case of the polluted waters, however, a somewhat longer period may intervene between the first appearance of gas and the numerical colon maximum, but in our experiments the latter generally occurred within twenty-four hours of the time of inoculation. This fact has a direct bearing on laboratory procedure. Since, in ordinary practice with the fermentation tube as applied to water examination, gas formation is expected if B. coli is present, and since streptococci are likely to appear soon after gas formation begins, it would seem advisable to look for B. coli in the confirmatory tests immediately after the first appearance of gas, rather than after a twenty-four to forty-eight hour period, as has been customary in some laboratories.

From the results of this investigation we suggest as a method for the detection of B. coli and sewage streptococci in water the following procedure:

Inoculate the desired quantity of water, preferably 1 c.c., into dextrose broth, in a tube or apparatus suitable for the ready determination of gas production. Incubate at 37° C.

After a few (six to twelve) hours' incubation examine the cultures for gas. Within two or three hours after the gas formation is first evident plate from the broth, into litmus lactose agar, incubating for twelve to eighteen hours at 37° C. If at the end of this time no acid-producing colonies are present, it is probably safe to assume that there were no colon bacilli present. If, on the other hand, red colonies are present, these must be further examined by the usual diagnostic tests for B. coli.

After the first plating from the dextrose broth replace the fermentation tube in the incubator, allow it to remain there for thirty-six hours, and then plate again in litmus lactose agar. This plating should give a nearly pure culture of streptococci, if these organisms were originally present in the water.

If colon bacilli are not found in the first set of plates, the streptococci may still be isolated, if present, by this method, or possibly after a somewhat shorter period of incubation than we have suggested.

A POSSIBLE RAPID TEST FOR STREPTOCOCCI.

Since the streptococci follow the colon bacilli in their development in the dextrose tube it is plain that they can easily withstand an amount of acid such as is produced in the tube by B. coli. We have taken advantage of this fact in attempting to devise a method for the detection of streptococci without waiting for colon bacilli to develop; since it seems desirable, if streptococci are to be reckoned with in the future as a part of the regular routine of bacteriological water analysis, to have this determination parallel with that of B. coli, and as rapid as possible. We have accordingly proposed to use for this purpose a medium of sufficiently high acidity to inhibit the growth of B. coli and of most water bacteria, but which shall still permit the development of streptococci.

The question, however, arises whether the desired result is to be determined by the kind of acid used or by the mere degree of acidity; *i. e.*, whether the effect is limited to the particular acid produced by colon bacilli, namely, lactic acid, or may be secured by the presence of a sufficient quantity of such acids as hydrochloric or sulphuric, as well as lactic. We have therefore determined by titration the approximate amount of acid produced in dextrose broth up to the period of maximum numbers of B. coli, and have prepared a dextrose broth of slightly greater acidity than that indicated by these titrations. On inoculation of such broth with fresh, raw sewage in great dilution, we have succeeded in cultivating streptococci in a nearly pure condition after only 24 hours' incubation. Further consideration of this problem is reserved for another paper.

In conclusion the authors wish to acknowledge with gratitude their indebtedness to Professor W. T. Sedgwick for helpful suggestions and his kindly interest in the work.

BIBLIOGRAPHY.

1. Dyar, H. G., and Keith, S. C., Jr. "Notes on Normal Intestinal Bacilli of the Horse, and of Certain Other Domestic Animals." *Tech. Quart.*, 1893, 6, 256.
2. Smith, T. "Ueber den Nachweis des Bacterium coli communis im Wasser." *Centr. f. Bakt.*, 1895, 17, 726.

2A. Flint, J. M. Notes on the Distribution of B. coli communis. *J. of the Am. Med. Assoc.*, 1896, 26, 410.

3. Belitzer. "Zur Lehre über das Bacterium coli communis." *Rev. Jahr. u. d. path. Mikr.*, 1899, 15, 326.
4. Moore, V. A. and Wright, F. R. "A Comparison of B. coli communis from Different Species of Animals." *J. Bost. Soc. Med. Sci.*, 1900, 4, 175.
5. Kruse, W. "Kritische und experimentelle Beiträge zur hygienischen Beurtheilung des Wassers." *Zeit. f. Hyg.*, 1894, 17, 1.
6. Weissenfeld, J. "Der Befund des Bacterium coli und das Thierexperiment sind keine brauchbaren Hülfmittel für die hygienische Beurtheilung des Wassers." *Ibid.*, 1900, 35, 76.
7. Prescott, S. C. "On the Apparent Identity of the Cultural Reactions of B. coli communis and Certain Lactic Bacteria." *Science*, 1902, 15, 363.
8. Prescott, S. C. "On Certain Precautions Required in Making and Interpreting the So-Called 'Colon Test' for Potable Waters." *Medicine*, 1903, 9, 20.
9. Papasotiriu, J. "Untersuchungen über das Vorkommen der Bact. coli in Teigmehl und Getreide, nebst einigen Bemerkungen über die Bedeutung der Bact. coli als Indikator für Verunreinigungen von Wasser und Fäkalien." *Archiv f. Hyg.*, 1902, 41, 204.
10. Freudenreich, E. von. "Ueber den Nachweis des Bacillus coli communis im Wasser und dessen Bedeutung." *Centr. f. Bakt.*, 1895, 18, 102.
11. Smith, T. "Water-Borne Diseases." *J. N. E. Waterworks Assn.*, 1896, 10, 203.
12. Laws and Andrewes. "Report on the Micro-Organisms of Sewage," *Report to the London County Council*, 1894, No. 216.
13. Houston, A. C. "Bacterioscopic Examinations of Drinking Water, with Particular Reference to the Relation of Streptococci and Staphylococci with Waters of This Class." *Ann. Rep. L. G. B.*, containing *Rep. of Med. Off.*, 1899; *Ann. Rep. L. G. B.*, containing *Rep. of Med. Off.*, 1900.
14. Horrocks, W. H. *Bacteriological Examination of Water.* London, 1901.

15. WINSLOW, C.-E. A. AND HUNNEWELL, MISS M. P. "A Study of the Distribution of the Colon Bacillus of Escherich and of the Sewage Streptococci of Houston in Polluted and Unpolluted Waters." *J. Med. Res.*, 1902, 3, 3, N. S., 502.

16. PRESCOTT, S. C., "A Note on Methods of Isolating Colon Bacilli." *Science*, 1902, N. S., 16, 408, 671.

17. GAGE, S. DEM. "Bacteriological Studies at the Lawrence Experiment Station, with Special Reference to the Determination of B. coli." *33d Ann. Rep. Mass. S. B. H.*, 1901.

18. LEGROS, F.-L. *Monographie des streptocoques et des agents des septicémies métadiphtériques, particulièrement des diplocoques.* Paris, 1902.